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Dear Dr. Stokes:

This public comment is delivered in response to Federal Register Notice Volume 74, Number 60, pages 14556-14557. It addresses the Summary Review Document (SRD), "Draft ICCVAM Summary Review Document: The Low Volume Eye Test", April 1, 2009.

The Summary Review Document purports to address the suitability of LVET data as an in vivo reference against which in vitro data might be compared. The analysis is central to the evaluation of the Draft Summary Review Document: Strategy for U.S. Environmental Protection Agency Ocular Hazard Classification and Labeling of Antimicrobial Cleaning Products Using *In Vitro* Alternative Test Methods. An SRD should demonstrate the high level of scholarship commensurate with its intended purpose. The completeness and veracity of the data presented and conclusions drawn are of interest to all of us working in the field of alternatives for the prediction of eye irritation in humans. A fundamental principle of scientific scholarship is the support of conclusion statements with data or reference to data. The reader may wish to review this SRD with that thought in mind. There are a number of key points in the SRD that might benefit from additional data and/or alternative interpretation. These points I should like to address in this public comment. The reader can then choose to include or ignore these additions as she or he feels appropriate. The points in question are repeated in several sections of this SRD. I will not try to address each occurrence but cite one representative passage. Each point begins with the specific text from the SRD followed by the comments.

1. "**Accidental eye injury is the leading cause of visual impairment in the U.S. and many of these injuries occur due to contact with workplace and household chemicals. According to the National Institute of Occupational Safety and Health, each day about 2000 U.S. workers have a job-related eye injury that requires medical treatment. Even more eye injuries occur in the home, with 125,000 eye injuries a year caused by accidents involving common household products such as oven cleaner and bleach (source American Academy of Ophthalmology).**"[lines 319-325] Eye irritation, from mild through severe, is a concern in the home and workplace, in sports and in military training. The overall incidence of accident-induced visual impairment is the result of mechanical injury, thermal burns, and chemical exposure. McGwin and collaborators report that the vast majority of eye injury come from mechanical trauma (i.e., contusions/abrasions and foreign body)[1]. What is more important to this SRD is the frequency of moderate to severe chemical injuries to the eye. Wagoner[2] has reviewed a series of published reports and concluded that alkali injuries (including those from certain high alkali household products) and to a lesser degree acid injuries are the primary chemical injuries observed in people. It is of interest that

personal-care, surfactant-based cleaning products (laundry, dishwashing, and the like) and household bleaches are not mentioned. A summary table alkali and acids materials most associated with human eye injury is redrawn from this reference and is provided in Attachment 1.

2. "**The majority of available LVET data were generated with surfactant-based mixtures or products which produce only a mild ocular irritant response or no response**"[280-281]…"**there is no information on the performance of known human corrosives in the LVET**"[285-286]. It is expected that the developer of the LVET would focus on product types within its portfolio. Looking at the types of products used in the home, surfactant-based cleaning products are common and so assessment of their eye irritation potential would be important. However, the final statement is quite surprising given the available literature. The pioneering mechanistic studies of Maurer and Jester [3-6] were performed using individual chemicals that included 37% formaldehyde, 8% NaOH, undiluted parafluoranaline, and 10% hydrogen peroxide. Griffith et al (1980) [7] used a series of chemicals to compare several instillation volumes (10, 30, 100 µL). With all three instillation volumes, several chemicals (29% SLS, 10% Acetic Acid, Calcium Hydroxide, (100%), and 38% Formaldehyde) produced severe damage that did not reverse in 21 days. NaOH, Acetic acid, and Calcium hydroxide are on the table provided in Attachment 1.
3. "**Gettings et al (1996) evaluated 25 surfactant formulations and their hazard classifications by the EPA and GHS, and reported several incidences of under prediction of an ocular corrosive or severe irritant in the Draize rabbit eye test by the LVET method.**"[281-284] The Cosmetics, Toiletries, and Fragrance Association (CTFA) produced some of the most useful data sets for the analysis of both the Draize and LVET in vivo tests as well as a wide range of in vitro assays. The Phase III work focused on surfactant-based formulations. All studies used the same batches of test material. Gettings et al (1996) [8] reported the Draize scores while Gettings et al (1998) [9] reported the low volume eye test scores. In their analysis of each data set, the authors used the Kay and Calandra (1960) [10] categories to assign degrees of irritancy potential. In both cases, the highest category assigned was Moderate. The EPA and GHS analysis was performed by others. Unfortunately, the GHS analyses (distribution of GHS categories) in tables 4-2 [435] and 4-4 [454] are incorrectly calculated. Products HZI (Skin Cleaner), HZK (Bubble Bath), and HZS (Shower Gel) produced lesions in one of the six animals treated in each group that did not recover by 21 days. Thus, these three test materials would be considered severe in the GHS scoring system. These errors in the GHS tables in turn impact some of the associated text [439-447]. Eye irritation categories obtained from a single in vivo assay are sometimes treated as absolutes, almost inherent properties of the test material (rather than properties of the test and associated regulatory interpretations/classification). The 6-rabbit test can be broken down into 20 unique combinations of 3 rabbits to model the current regulatory test. This type of bootstrap analysis provides some insight into the potential irritation categories that might be obtained with the test material (Attachment 2).
4. **"…comparative human data from clinical studies and accidental exposures proposed to support its accuracy are largely with substances that are mild or non-irritating. Ethical considerations have limited the severity of substances that can be tested in human clinical studies. Such data provide little assurance to the regulatory**

agencies charged with protecting public health that the LVET can provide adequate protection from substances that may cause moderate or severe ocular injuries in humans."[lines 304-310], all of **Section 5.0 Performance of the LVET vs. the Draize Rabbit Eye Test Considering Human Study Data and Experiences**"[lines 458-493] and "**Accidental exposures are not generally considered to be a reliable source of the true ocular hazard potential since such exposures are likely immediately follows by flushing the eyes with large volumes of water.**"[lines 594-597] Both the Draize and LVET assays are intended to address eye irritation potentials from non-irritating through stages to severe. Laying the basis, through clinical trials, to show the LVET (or other assays) as an effective predictor of irritation in the milder end of the spectrum seems quite appropriate. I hope most readers would also agree that clinical trials with severe eye irritants are both unethical and largely unnecessary. As mentioned before, we have a large body of data on severe (vision impairing) damage from accidental chemical exposure. Thus it is very surprising see the use of such data so roundly criticized by the NICEATM. The statements regarding the appropriateness of using epidemiological data (accidental exposure) seemed to have originated from the NICEATM as they supported neither by data nor reference from the ophthalmic literature. Together, these sections propose that a test designed to identify the degree of irritation potential of a test material and thus mitigate the risks from its accidental to humans cannot be calibrated or verified based upon the decades of human use and accidental exposure. A single or even small number of accidental exposures might not provide a robust picture of the human irritation potential. However, the National Electronic Injury Surveillance System (NEISS) database contains hundreds of reports over a wide range of product/chemical classes. From Appendix 11 of the International Association for Soaps, Detergents and Maintenance Products extensive BRD (Appendix A of the SRD), the 1980 to 1991 data are available for several kinds of cleaning product categories. In all cases, the exposed individual was seen by an emergency department. Here are several examples: Laundry soaps and detergents (230 exposures and all evaluated/treated and released), Dishwashing liquids (90 exposures and all evaluated/treated and released), Fabric treatments (30 exposed and all evaluated/treated and released), General purpose household cleaners (664 exposed and all evaluated/treated and released) and Household Bleaches (often with other cleaning products) (961 exposed and all but 4 evaluated/treated and released [the final disposition of these 4 individuals was not available from the data presented]). These data from emergency departments do not address specific products but do provide a strong sense of the irritation character of product classes. The somewhat higher irritation potential of the bleaches is consistent with the results of Maurer et al (2001) [3] using the LVET with 13% sodium hypochlorite. In this study, recovery extended past 7 days making this concentration of bleach an EPA Category II. The point here is very simple. To dismiss the use of epidemiological data for eye irritation is to fly in the face of rational science and the considerable efforts to identify and characterize human risk (including those efforts of the Consumer Product Safety Commission). To ignore these data is to reduce the current and future assessment of eye irritation to a matter of dogma (an un-testable belief) rather than data (a testable hypothesis).

5. "**In contrast, there are no documented instances where a substance with a hazard category determined in the Draize eye test produced a more severe hazard category response in humans following accidental exposure or ethical human studies**."[lines

314-317] This statement (assertion) has appeared in NICEATM-derived BRDs since 2004 and has yet to ever be supported by data. In assessing the validation (and appropriateness for regulatory use) of new tests, both the sensitivity and specificity are evaluated. Acceptable predictive capacity is found in the ability to identify both positive and negative responses relative to the reference test (or species of interest). In the ICCVAM evaluation of the four in vitro methods for the prediction severe eye irritants, this point was reaffirmed. The SRD statement above might be substantiated at some point by data, but even so, it refers only to sensitivity and ignores the need for specificity. It is the matter of specificity that makes the data from Gettings et al (1996 and 1998) so important. All of us have direct experience using such consumer products. To see the likes of gel cleaner, shampoo, and facial cleaner placed in the same hazard category as concentrated hydrofluoric acid, formaldehyde, sulfur mustard, and sodium hydroxide gives one pause. Where is the specificity? One is reminded on the Aesop's fable of the Sheppard Boy and the Wolf (Attachment 3). Specificity is the key to credibility.

I thank you for the opportunity to make this public comment and ask that it be made available to the Expert Panel and general public before the 19-21 May 2009 meeting. I also look forward to attending the Peer Review Panel meeting.

Sincerely yours,

/s/

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References

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Attachment 1

Common Causes of Chemical Injury*

Class	Compound	Common Sources/Use	Comments
Alkali	Ammonia [NH_3]	Fertilizer	Combines with water to form NH_40H fumes
		Refrigerants	Very rapid penetration
		Cleaning agents (7% solution)	
	Lye [NaOH]	Drain cleaner	Penetrates almost as rapidly as ammonia
	Potassium hydroxide [KOH]	Caustic potash	Similar to that of lye
	Magnesium hydroxide [$Mg(OH)_2$]	Sparklers	Produces combined thermal and alkali injury
	Lime [$Ca(OH)_2$]	Plaster	Most common cause of chemical injury in the work place
		Mortar	Poor penetration
		Cement	Toxicity increased by retained particulate matter
		Whitewash	
Acids	Sulfuric acid [H_2SO_4]	Industrial cleaners	Combines with water to produce corneal thermal injury
		Battery acid	May be associated with foreign body or laceration from batter acid
	Sulfurous acid [H_2SO_3]	Formed from sulfur dioxide (SO_2) by combination with corneal water	Penetrates more easily than other acids
		Fruit and vegetable preservatives	
		Bleach	
		Refrigerants	
	Hydofluoric acid [HF]	Glass polishing	Penetrates easily
		Glass frosting	Produces severe injury
		Mineral refining	
		Gasoline alkylation	
		Silicone	

		production	
	Acetic acid [CH_3COOH]	Vinegar 4-10%	Mild injury with less than 10% contamination
		Essence of vinegar 80%	Severe injury with higher concentration
		Glacial acetic acid 90%	
	Chromic acid [Cr_2O_3]	Used in the chrome plating industry	Chromic exposure produces chromic conjunctivitis with brown discoloration
	Hydrochloric acid [HCl]	Used as a 31-38% solution	Severe injury only with high concentration and prolonged exposure

- Redrawn from Wagoner, 1997 [2]

Attachment 2

The Draize and LVET eye irritation determinations were performed on 6 rabbits per test material per method using coded samples and a random block design. The studies were GLP compliant. The results from the 6 rabbits in each test group can be distributed into 20 unique combinations of 3 rabbits. Three rabbits are now the standard for the Draize and LVET assays. One can then compare the irritation category for each of the 20 combinations. Below are shown the original 6-rabbit category and the distribution of the categories of the 20 3-rabbbit categories. Only the EPA categories are shown for simplicity. These data illustrate the potential for rather disparate predictions when only one or two animals fail to recover among the six treated (see for example the Draize results for HZD or LVET results for HZI).

Draize Test (Gettings et al, 1996)

Code	Name	6-rabbit category	Distribution of 3-rabbit categories				# not cleared[1]	Average days to clear[2]
			EPA Cat I	EPA Cat II	EPA Cat III	EPA Cat IV		
HZA	Shampoo 7	I	16	4	0	0	2	12.3
HZB*	Liquid Soap 1	III	0	0	20	0	0	4.0
HZC*	Shampoo 1	III	0	0	20	0	0	5.2
HZD*	Shampoo 5	III	0	0	20	0	0	3.7
HZE	Gel Cleaner	I	10	0	10	0	1	4.2
HZF	Baby Shampoo 2	I	16	4	0	0	2	10.5
HZG*	Shampoo 8	III	0	0	20	0	0	3.5
HZH	Eye Makeup remover	IV	0	0	0	20	0	1.0
HZI	Skin Cleaner	I	19	1	0	0	3	9.3
HZJ	Mild Shampoo	IV	0	0	0	20	0	1.3
HZK	Bubble bath	I	20	0	0	0	5	7.0
HZL	Foam Bath	I	19	0	1	0	3	7.0
HZM*	Shampoo 3	III	0	0	10	10	0	2.3
HZN*	Shampoo 6	III	0	0	20	0	0	2.8
HZP	Baby Shampoo 1	III	0	0	19	1	0	2.7
HZQ	Cleaning Gel	III	0	0	20	0	0	3.5
HZR*	Facial Cleansing Foam	I	10	0	10	0	1	5.2
HZS	Shower Gel	I	19	1	0	0	3	9.3
HZT	Polishing Scrub	IV	0	0	0	20	0	1.0
HZU*	Hand Soap	III	0	0	20	0	0	4.5
HZV*	Shampoo 4	III	0	0	20	0	0	3.7
HZW*	Liquid Soap 2	III	0	0	20	0	0	6.0
HZX	Shampoo 2	I	16	4	0	0	3	9.3
HZY	Shampoo AntiD	I	16	4	0	0	2	12.3
HZZ	Facial Cleaner	IV	0	0	0	20	0	1.0

*Diluted to 25% in water before testing

[1] Number of animals that did not recover by 21 days

[2] The average number of days to clear in those animals that did clear by 21 days

LVET (Gettings et al, 1998)

Code	Name	6-rabbit category	Distribution of 3-rabbit categories				# not cleared[1]	Average days to clear[2]
			EPA Cat I	EPA Cat II	EPA Cat III	EPA Cat IV		
HZA	Shampoo 7	III	0	0	20	0	0	2.0
HZB*	Liquid Soap 1	IV	0	0	0	20	0	0.0
HZC*	Shampoo 1	III	0	0	20	0	0	2.0
HZD*	Shampoo 5	III	0	0	16	4	0	0.8
HZE	Gel Cleaner	III	0	0	20	0	0	1.3
HZF	Baby Shampoo 2	III	0	0	20	0	0	2.8
HZG*	Shampoo 8	III	0	0	19	1	0	1.3
HZH	Eye Makeup remover	IV	0	0	0	20	0	0.0
HZI	Skin Cleaner	I	10	0	10	0	1	3.8
HZJ	Mild Shampoo	IV	0	0	0	20	0	0.0
HZK	Bubble bath	I	10	0	10	0	1	4.2
HZL	Foam Bath	III	0	0	20	0	0	3.5
HZM*	Shampoo 3	III	0	0	19	1	0	1.0
HZN*	Shampoo 6	III	0	0	16	4	0	0.7
HZP	Baby Shampoo 1	III	0	0	10	10	0	0.3
HZQ	Cleaning Gel	IV	0	0	0	20	0	0.0
HZR*	Facial Cleansing Foam	III	0	0	16	4	0	0.7
HZS	Shower Gel	I	10	0	10	0	1	5.4
HZT	Polishing Scrub	IV	0	0	0	20	0	0.0
HZU*	Hand Soap	III	0	0	16	4	0	0.8
HZV*	Shampoo 4	III	0	0	10	10	0	0.3
HZW*	Liquid Soap 2	III	0	0	20	0	0	2.3
HZX	Shampoo 2	III	0	0	20	0	0	4.2
HZY	Shampoo AntiD	II	0	10	10	0	0	6.2
HZZ	Facial Cleaner	IV	0	0	0	20	0	0.0

*Diluted to 25% in water before testing

[1] Number of animals that did not recover by 21 days

[2] The average number of days to clear in those animals that did clear by 21 days

Attachment 3

Many of us will remember the short fables of Aesop from childhood. Fables tend to be a bit dramatic with morals directed to the proper upbringing of children. Thus, the moral here should not be over interpreted to the subject at hand. The importance of this fable is to remind us of the importance of raising the alarm only for real danger least all alarms be ignored.

"The Boy Who Cried Wolf, also known as *The Shepherd Boy and the Wolf*, is a fable attributed to Aesop (210 in Perry's numbering system[1]). The protagonist of the fable is a bored shepherd boy who entertained himself by calling out "Wolf!". Nearby villagers who came to his rescue found that the alarms were false and that they had wasted their time. When the boy was actually confronted by a wolf, the villagers did not believe his cries for help and the wolf ate the flock (and in some versions the boy). The moral is stated at the end of the fable as:

Even when liars tell the truth, they are never believed. The liar will lie once, twice, and then perish when he tells the truth."

(From: http://en.wikipedia.org/wiki/The_Boy_Who_Cried_Wolf)